with high troughs ≥2 mcg/mL had enhanced platelet recovery (p=0.005). In a competing risk 2-week landmark analysis, there were no differences grade II-IV aGVHD incidences (61% vs 57%, p=0.52) according to troughs. However, patients with a low MPA trough early post-CBT had nearly triple the incidence of grade III-IV aGVHD (27.8% vs 9.5%, p=0.06, Figure).

Conclusions: Higher total MPA troughs are safe and may protect against severe aGVHD. The platelet benefit could be explained by the lower severe aGVHD incidence. Prospective investigation of MPA troughs, and ultimately intervention based on drug monitoring in CBT recipients is warranted.

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Inhibition of Cdk2 Inactivates EZH2 and Induces Epigenetic Regulation of Fopx3 Leading to the Generation of CD8+ Treg and Protection from GvHD

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In spite of intense efforts, control of graft versus host disease (GvHD) remains incomplete and novel therapeutic approaches are required. Cdk2 has a central role in cell cycle re-entry of mature T lymphocytes and inhibition of Cdk2 is mandatory for induction of T cell anergy in vitro and tolerance in vivo. To determine the effects of Cdk2 inhibition on GvHD, we used the B6D2F1 mouse model of allogeneic BMT and two different Cdk2 inhibitors (Cdk2i), CYC202 and CYC205. Lethally irradiated B6D2F1(K^d) recipients were infused with bone marrow from C57BL/6(K^b) donors with (BMT) or without splenocytes and were subsequently treated with each Cdk2i for three weeks. Treatment was administered daily during week 1, every other day on week 2, and twice a week on week 3 followed by assessment of GvHD during a 70-day period. BMT recipients treated with Cdk2i displayed a transient weight loss and subsequently regained weight to levels comparable to controls. Treated BMT recipients also displayed delayed GvHD mortality (p=0.0054). Treg have a central role in mediating protection from GvHD. To examine whether Cdk2i induced Treg, we used GFP^- T cells from Foxp3.GFP-KI mice as a source of T cells. Assessment of peripheral blood lymphocytes, splenocytes, lymph nodes and intestinal lymphoid cells (ILC) in treated and control BMT recipients revealed no differences in $CD4^+GFP^+$ Treg. In contrast, $CD8^+GFP^+$ Treg were increased in the treated group, predominantly in ILC, which displayed a 5-fold increase of $CD8^+$ Treg (p=0.05). To investigate the mechanisms via which Cdk2i had a selective effect on $CD8^+$ Treg, we isolated $CD4^+GFP^-$ and $CD8^+GFP^-$ T cells from Foxp3.GFP-KI mice and subjected them to in vitro Treg polarization. Cdk2i had almost no effect on $CD4^+GFP^+$ cells but induced a 2-4 fold increase of $CD8^+GFP^+$ cells. Culture of $CD8^+GFP^-$ cells with stable concentrations of Cdk2i and decreasing concentrations of TGF-β revealed that Cdk2i induced $CD8^+$ Treg differentiation in the presence of TGF-β concentrations that failed to induce $CD8^+$ Treg cells when used alone. Expression of FOX family genes is regulated by transcriptional and epigenetic mechanisms. A critical epigenetic regulator of FOX transcription factors in cancer cells is the Polycomb group (PcG) protein, enhancer of zeste homologue 2 (EZH2), which promotes histone H3 lysine 27 trimethylation (H3K27me3) and induces epigenetic gene silencing. Cdk1 and Cdk2 phosphorylate EZH2 at Thr350 in an evolutionarily conserved motif. Phosphorylation of Thr350 is important for EZH2 recruitment and maintenance of H3K27me3 levels at EZH2-target loci. Upon polarizing $CD8^+$ T cell culture, EZH2 displayed robust phosphorylation on Thr350, which was blocked by Cdk2i. This event temporally coincided with a 44-fold increase in Foxp3 mRNA expression compared to control T cells. These results reveal an unexpected mechanism via which Cdk2 inhibitors induce $CD8^+$ Treg and protection from GvHD.

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IL-22 Administration Protects Intestinal Stem Cells from Gvhd

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Factors regulating damage and regeneration of the intestinal epithelium after allogeneic BMT are poorly understood. We have previously shown that IL-22 produced by recipient-derived innate lymphoid cells (ILCs) provides a critical signal for epithelial recovery following experimental BMT. However, intestinal IL-22 levels are reduced in GVHD due to the elimination of radioresistant host ILCs. We therefore sought to determine if IL-22 administration post-BMT could negate the effect of ILC elimination and reduce GVHD pathology. We utilized a clinically modeled LP into C57BL/6 (B6) minor antigen mismatched model with T cell-depleted marrow and purified T cells transplanted into lethally irradiated mice.

We found that daily administration of rIL-22 (4ug IP starting day +7) led to decreased GVHD pathology in recipient small and large intestine three weeks post-BMT (p<0.001). Further assessment of the intestinal pathology indicated that recipients of rIL-22 had decreased intestinal crypt apoptosis in both small and large intestine (p<0.01) with no difference in intestinal and splenic lymphocytes or inflammatory cytokine levels.

To assess the effects of IL-22 administration on the intestinal stem cell (ISC) compartment, we performed LP into B6 allo-HCT using Lgr5-LacZ ISC reporter mice. Recipients treated with rIL-22 demonstrated increased numbers of Lgr5+ ISC three weeks post-HCT during active GVHD with no immunosuppression (p<0.05). Furthermore, we found increased ISC Ki-67 expression in Lgr5-GFP reporter mice with GVHD after IL-22 treatment, indicating increased ISC proliferation in response to IL-22.

In addition to Lgr5+ cells, it has been reported that BMI-1+ crypt cells may possess ISC activity after crypt damage. Crypt cells from BMI-1-GFP reporter mice were indeed found to be IL-22R+ at baseline (7-10% IL-22R surface expression). However, BMI-1 mRNA expression in small intestine of mice with GVHD was not affected by IL-22 administration, suggesting that the effect of IL-22 administration in vivo was not due to stimulation of BMI-1+ cells. Additionally, there was no difference in Wnt3 or EGF expression, arguing that improved ISC survival after IL-22 administration was not due to improvement in ISC niche function. In contrast, IL-22 treatment demonstrated increased Reg3γ (p<0.001) and Reg3β (p<0.01) expression, suggesting a potential antimicrobial benefit of IL-22 administration.

In summary, we found that IL-22 administration could reduce intestinal pathology and improve ISC recovery in GVHD. This appeared to be due to direct stimulation of Lgr5+ ISCs, and not due to improved support of the ISC niche. These

results suggest that post-transplant IL-22 administration represents a novel strategy to reduce gut GVHD by direct protection of intestinal epithelium without limiting immune function post-transplant.

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Targeting Sag in Donor T Cells As a Novel Strategy for Reducing Gvhd

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Neddylation is crucial for the degradation of certain proteins. However its role in regulating T cells is unknown. Neddylation is mediated by cullin-RING ligase (CRL) protein complex, an E3 ubiquitin ligase and its critical adapter element, SAG protein (sensitive to apoptosis gene protein). We explored the role of SAG and thus neddylation in T cells by utilizing two different, but complementary approaches, namely, genetic knock-out and chemical inhibition with small molecule. The T cell specific SAG KO animals were generated by crossing B6 $SAG^{fl/fl}$ mice with B6 LCK-Cre mice. The KO animals were viable. The splenic and thymic analyses showed no significant differences in the numbers of conventional T cells (Tcons) and Tregs between the KO and WT animals. In vitro functional analysis of Tcons, however, revealed that stimulation with either allogeneic splenocytes or by α-CD3 and α-CD28 antibody, $SAG^{-/-}$ T cells showed significantly decreased proliferation ($P<0.002$). Phenotypic analysis following stimulation demonstrated that $SAG^{-/-}$ T cells showed reduced expression of CD69, CD44 and greater expression of CD62L when compared to WT-T cells ($P<0.04$). The KO-T cells also demonstrated reduced expression of T effector signature cytokines, IL-17, IFN-γ and IL-4. Similar reduction in proliferation, activation marker expression and release of cytokines was observed when the WT-T cells were treated with small molecule inhibitor of neddylation, MLN4924.

We next determined the in vivo relevance of SAG and neddylation in Tcons by utilizing the MHC disparate (B6→BALB/c) model of allogeneic BMT. The BALB/c animals were lethally irradiated and transplanted with TCD BM from either syngeneic or allogeneic WT-B6 animals along with $5x10^5$ splenic T cells from either the WT B6 or $SAG^{-/-}$ B6 animals. The allogeneic animals that received $SAG^{-/-}$ T cells demonstrated markedly reduced clinical GVHD and significantly increased survival when compared to those that received WT-B6 T cells ($P<0.001$). Similar results were observed in B6→B6D2F1 model. To further confirm our results and to determine potential translational application, we utilized the small molecule MLN4924, once again in the B6→BALB/c system. The recipient mice were lethally irradiated and received 5 doses of MLN4924 (20mg/kg, day-1 to day +3 of BMT) along with WT-B6 T cells. Mice receiving MLN4924 demonstrated significantly decreased clinical GVHD and improved survival. Our studies thus demonstrate that SAG is a novel molecular target for regulating T cell responses and mitigating GVHD. Furthermore, the clinical availability of the small molecule, MLN4924, suggests that this strategy could be tested in carefully designed human clinical trial for attenuating GVHD.

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Sensitization to HY-Antigen in Female Donors Was Not Associated with the Post-Transplant HY-IgG Development Nor Clinical Outcomes in Sex-Mismatched Transplantation

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Background: Transplant of male recipients from female donors (F>M HCT) is well known as a risk factor for developing chronic graft-versus-host disease (cGVHD). We have so far suggested that B cell response against minor histocompatibility antigens encoded on the Y chromosome, called H-Y antigens, develops following F>M HCT and associates with cGVHD. Here, we hypothesize that pre-sensitization to HY-antigen in a female donor may affect the post-HCT HY-IgG development and clinical outcomes following F>M HCT. This study uses our novel HY microarray to determine the prevalence and impact of donor HY-IgG.

Methods: We measured IgG against 5 HY antigens (DBY, UTY, ZFY, EIF1AY, & RBS4Y) in 289 female donors (age:18-60) of high resolution 8/8 HLA-matched HCT facilitated by the NMDP between 1990-2002 and assessed the impact of HY seropositivity on cumulative cGVHD incidence and other clinical outcomes.

In addition, we studied 90 Stanford adult female donors and their corresponding male recipients between 2005 and 2012 who survived without relapse for at least 3m post-HCT and assessed the association of HY-IgG development between pre- and post-HCT. The cut-off value for seropositivity was defined as Q3 + 2xIQR, determined from plasma of 60 maledonors. HY-score was defined as the cumulative number of targeted HY antigens.

Results: Prevalence of HY-IgGs in female donors is shown in Table1. Half of female donors had at least one of 5 HY-IgG(s). Univariate analyses of NMDP cohort showed that individual HY-IgGs in female donors were not associated with cGVHD. Focusing on increasing HY-score, we did not detect association with cGVHD nor other clinical outcomes (Table 2). This absence of association was also observed in Stanford cohort. Further, we were unable to show the

Table 1

	DBY	UTY	ZFY	EIF1AY	RPS4Y	Any-HY
NMDP (n=289)	63 (22%)	112 (39%)	22 (8%)	7 (2%)	42 (15%)	143 (49%)
Stanford (n=90)	20 (22%)	32 (36%)	4 (4%)	1 (1%)	8 (9%)	46 (51%)

Table 2

(NMDP)	cGVHD		aGVHD		Relapse		TRM		OS	
HY-score	HR	P	HR	P	HR	P	HR	P	HR	P
0 (n=146)	1	-	1	-	1		1	-	1	-
1 (n=75)	1.34	0.15	1.17	0.46	1.22	0.54	0.85	0.44	0.98	0.89
2 (n=39)	1.12	0.65	1.25	0.39	0.63	0.31	0.62	0.078	0.81	0.39
3 to 4 (n=29)	1.32	0.37	1.64	0.064	1.85	0.19	1.3	0.34	1.48	0.099